

Mismatch scanning Assay. (Endo V / DNA Ligase)

1. PCR amplify gene using primers with different fluorescent labels and *Taq* DNA polymerase.

2. Denature and reanneal PCR products to form heteroduplexed DNA. (Homoduplexed products not shown).

3. Preferentially nick DNA one base to the 3' side of mismatches using thermostable Endonuclease V.

4. Add thermostable ligase to re-seal background nicks at perfect match regions.

5. Separate fluorescent products on a DNA sequencer (using length standards) to approximate site of mismatch.

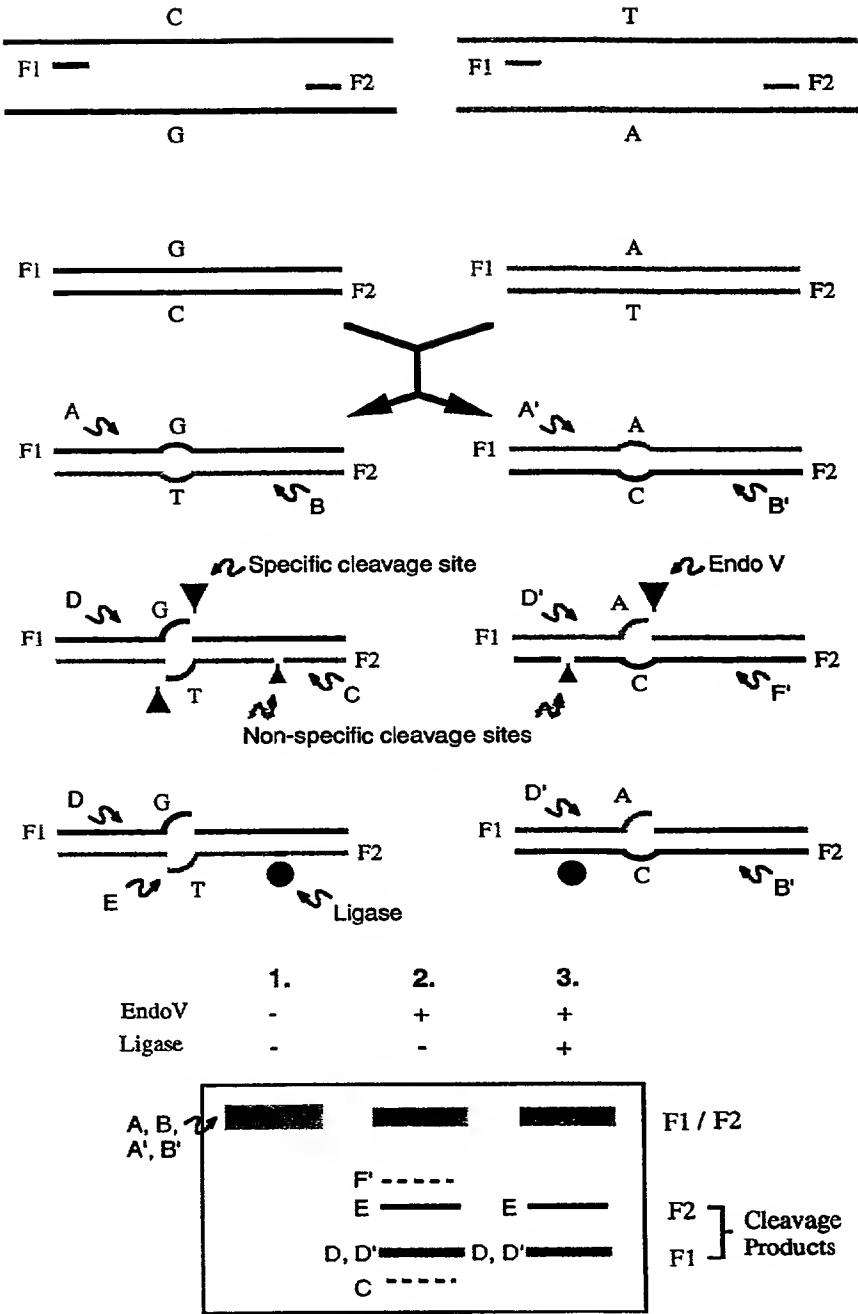


Figure 1

75°C 15'

Crude extract

SP Column

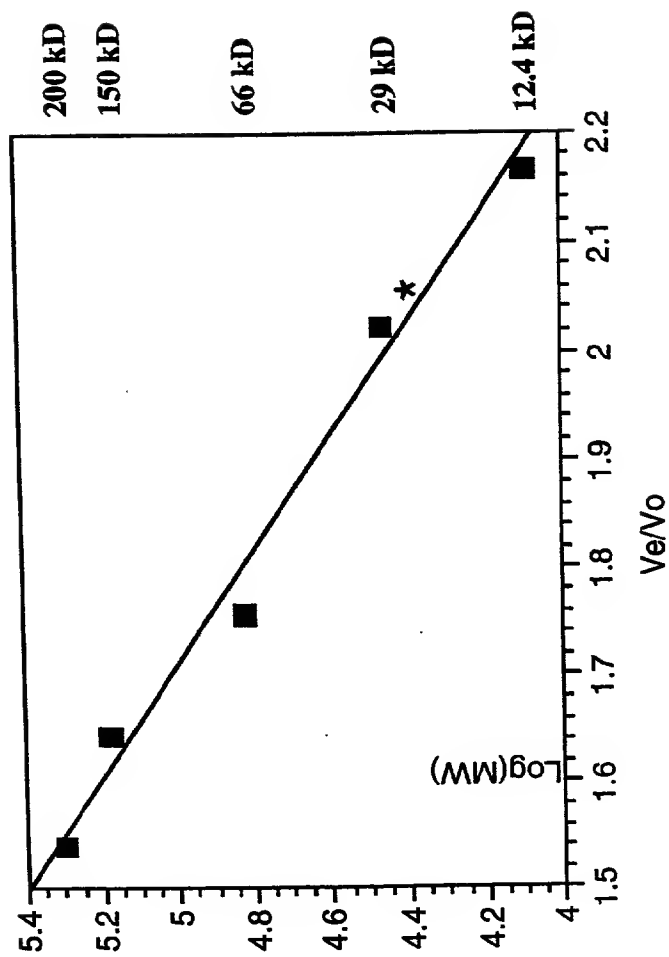
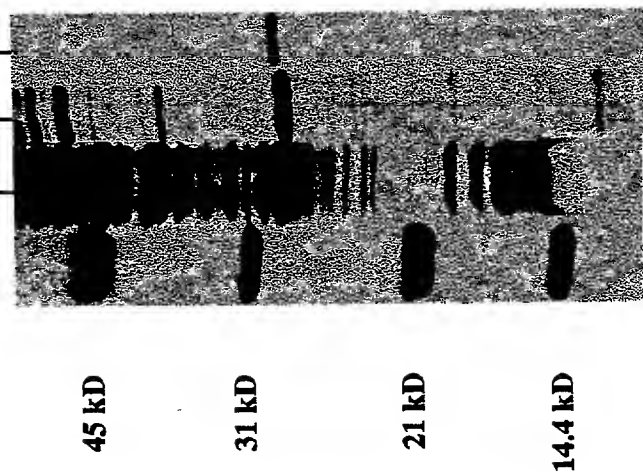


Figure 2

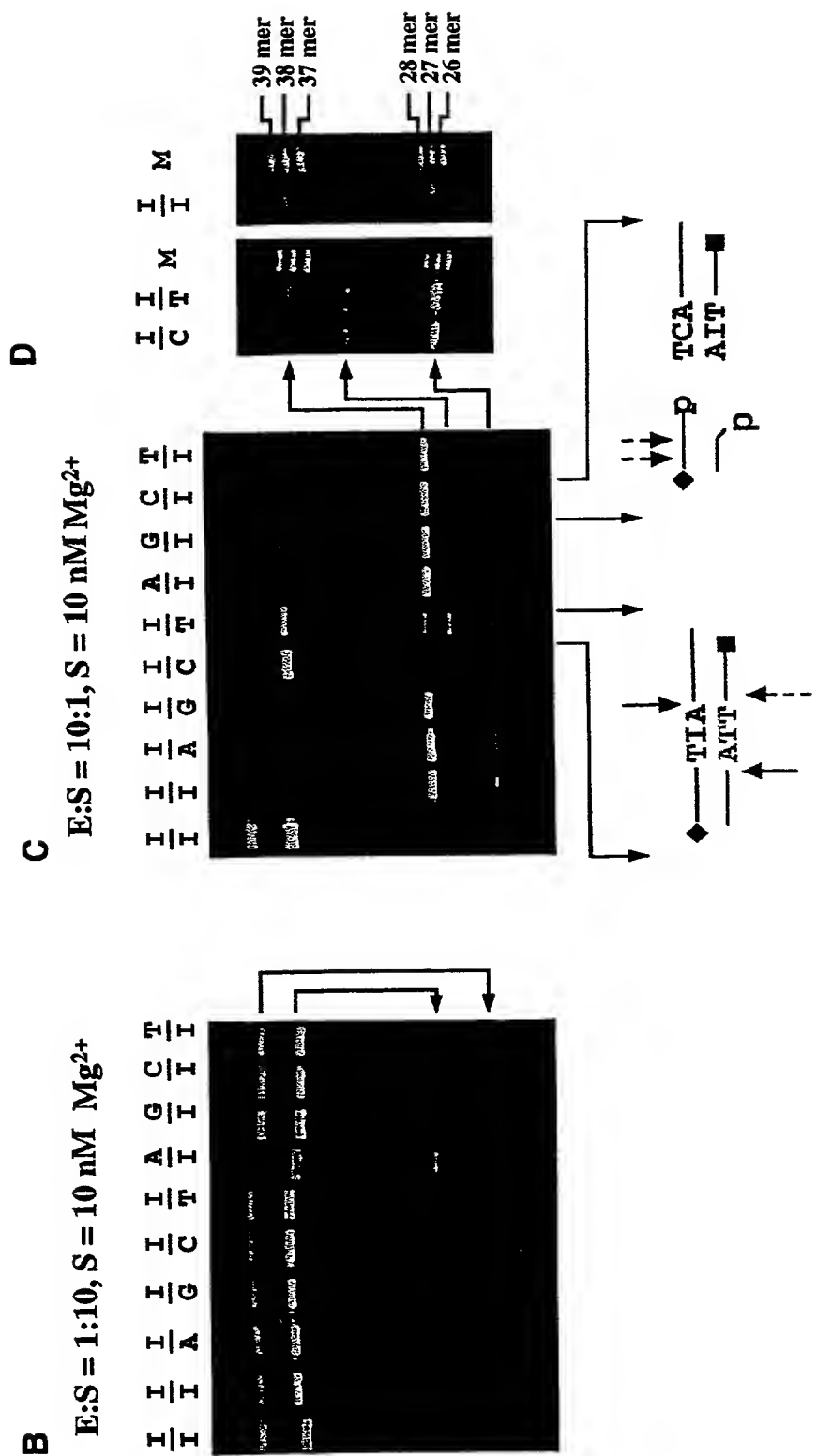
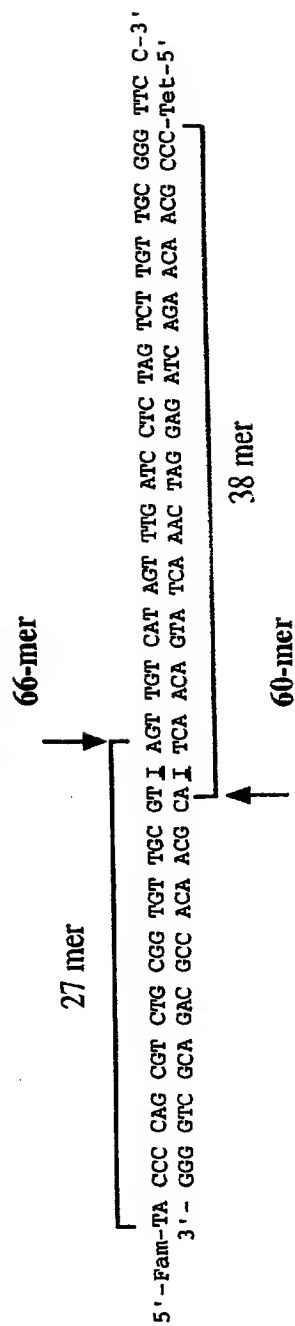
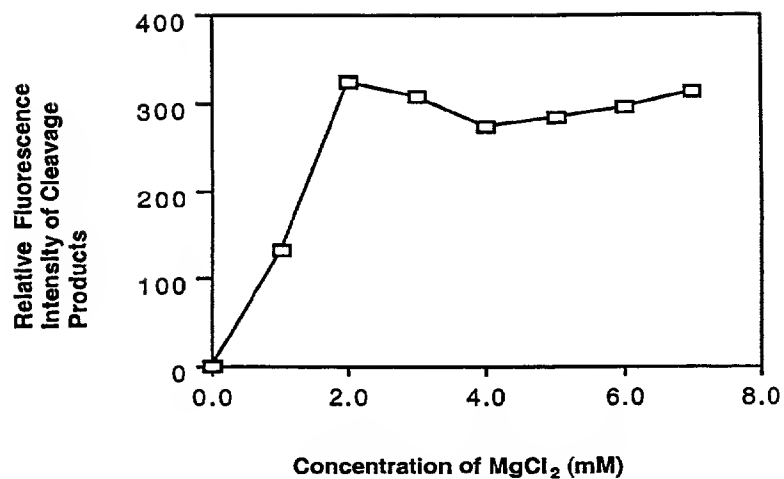


Figure 3

A



B

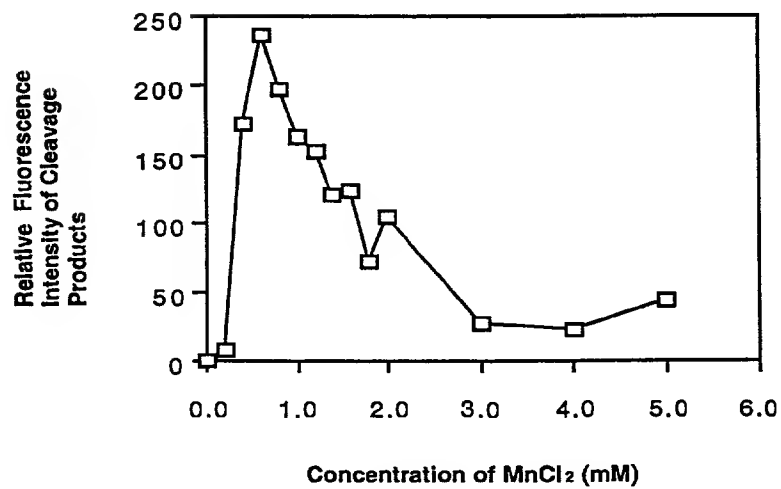


Figure 4

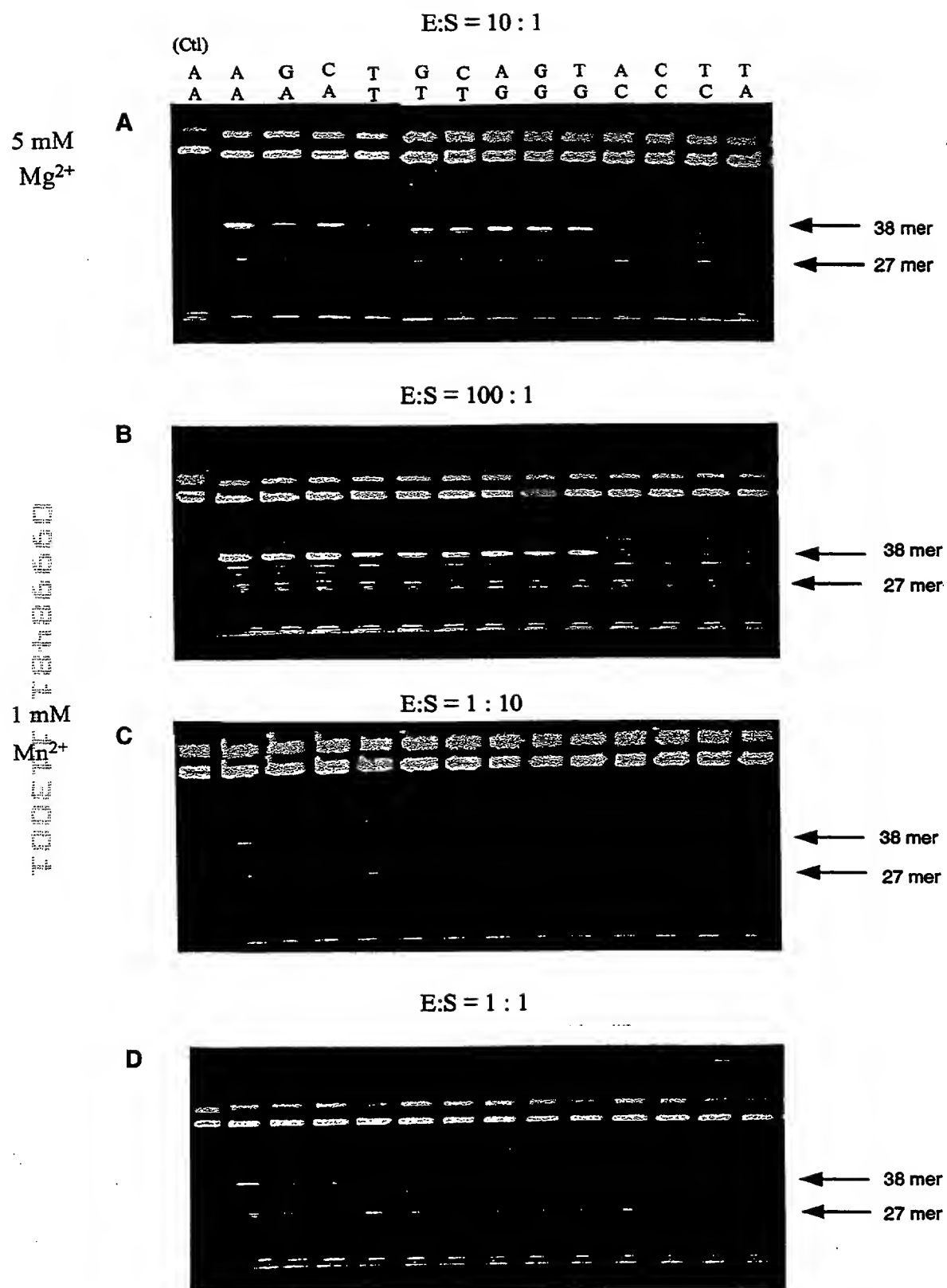


Figure 5

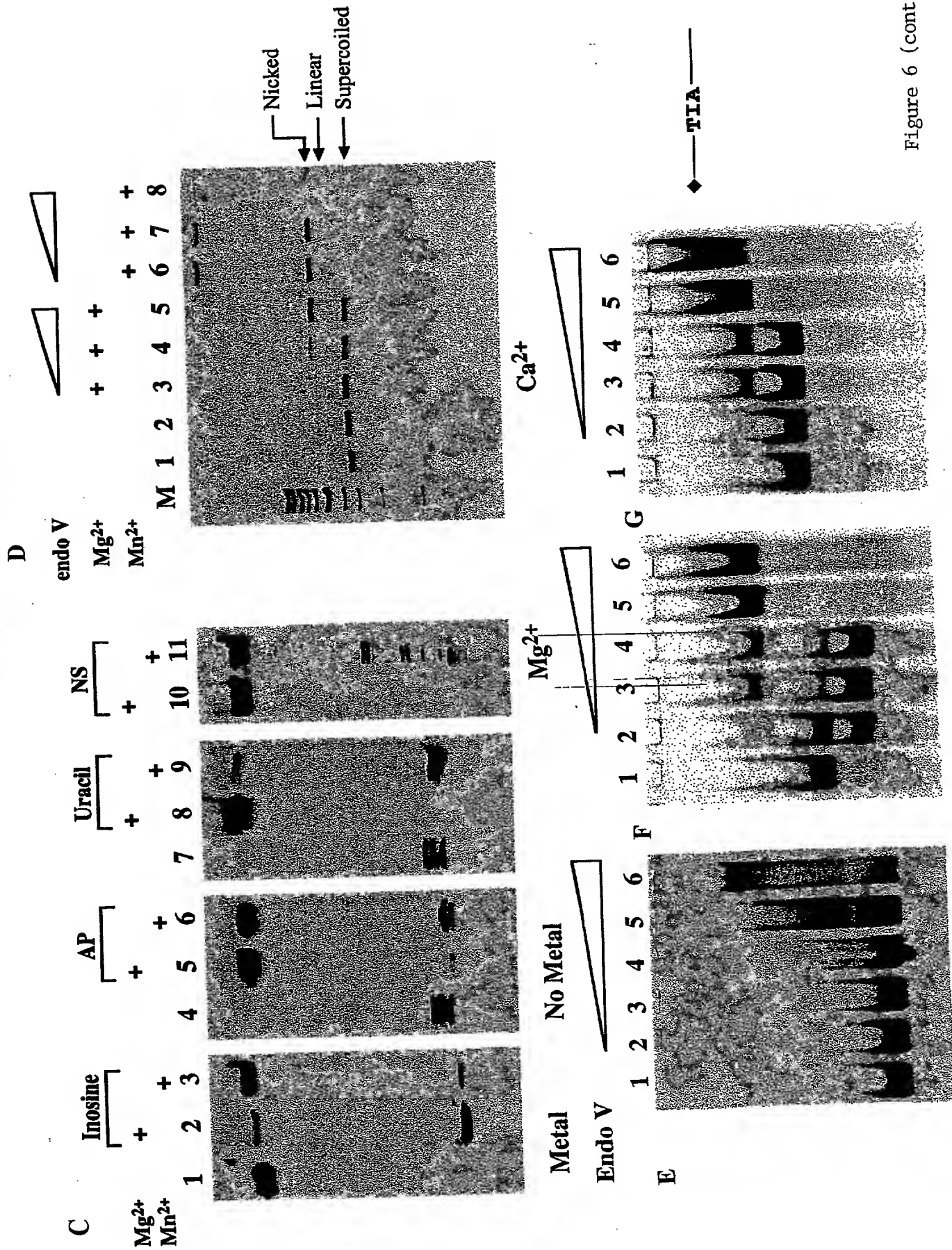


Figure 6 (cont.)

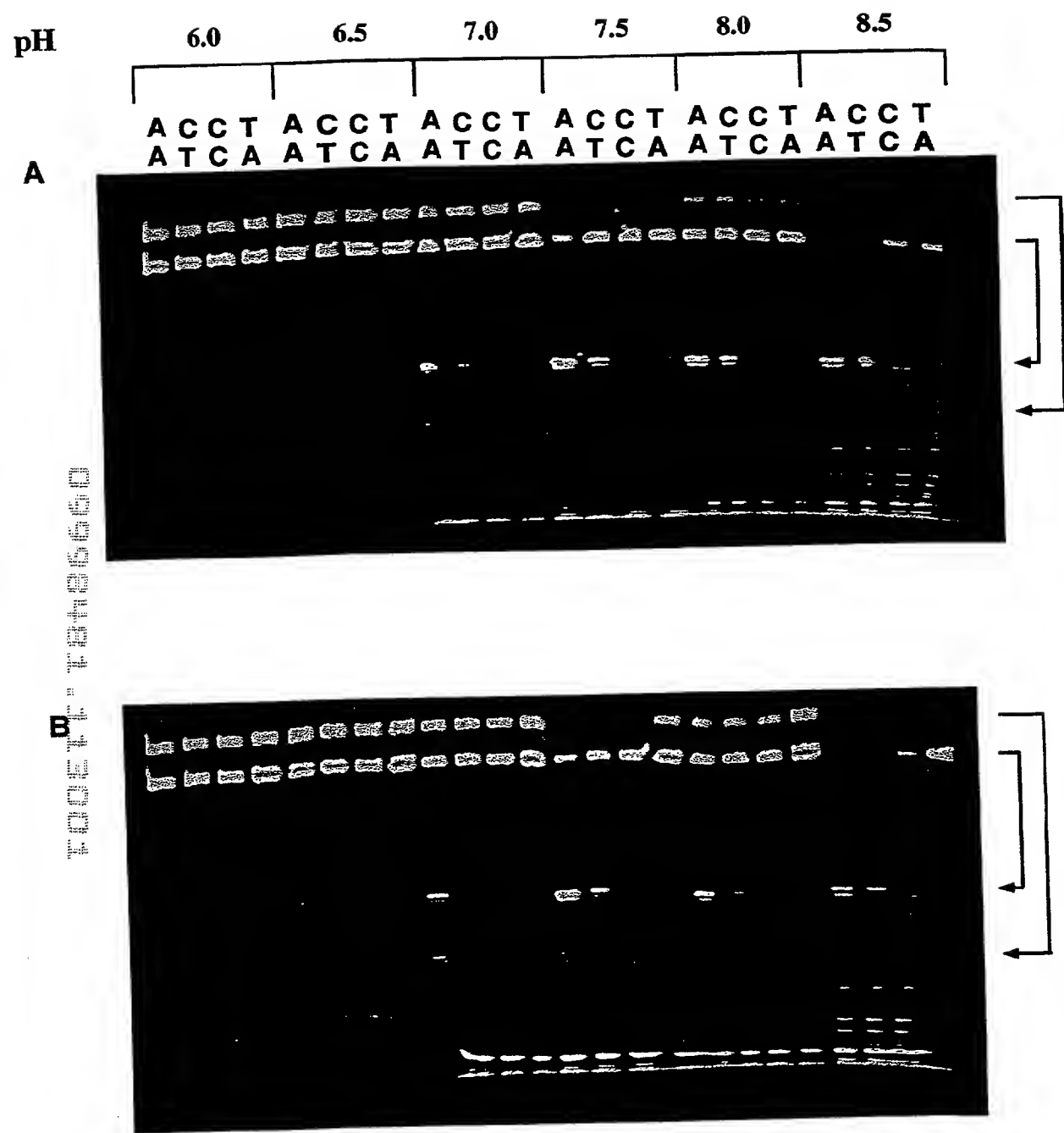


Figure 7.

NaCl/mM

0

50

100

150

200

250

A C C T A C C T A C C T A C C T A C C T A C C T A C C T A

A T C A A T C A A T C A A T C A A T C A A T C A A T C A

 Mg^{2+}

M

 Mn^{2+}

Figure 8

Kras G12V (G->T)

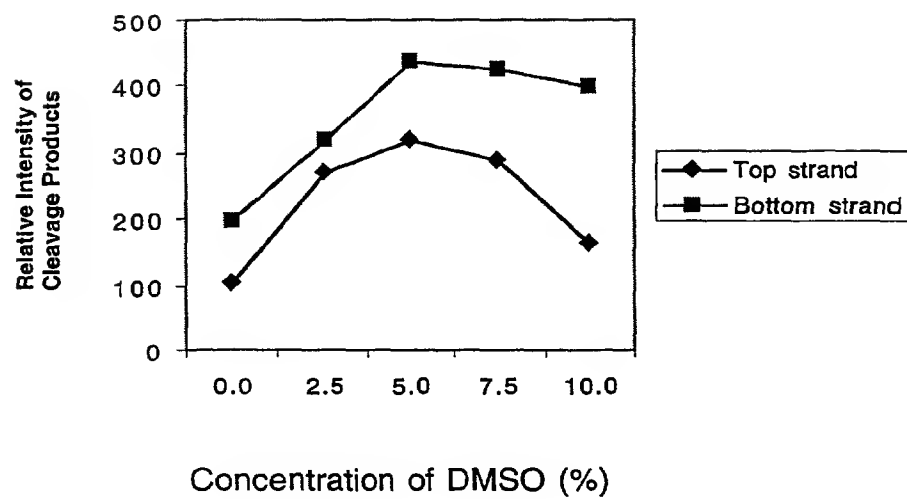
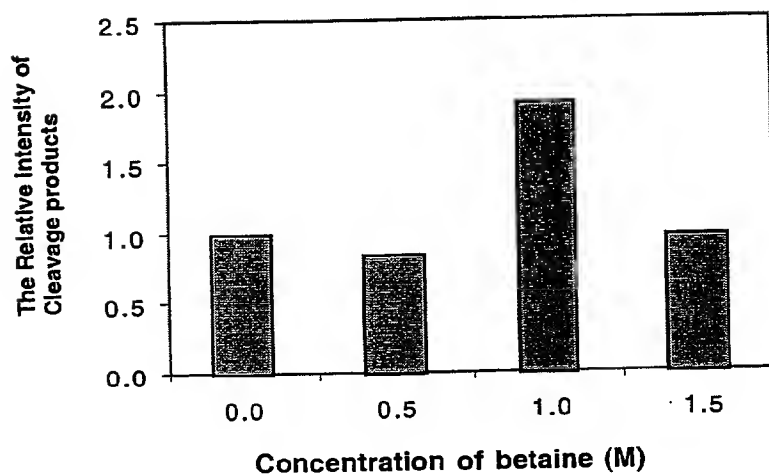


Figure 9

A

APC I1307K(T->A)



B

Kras G12V (G->T)

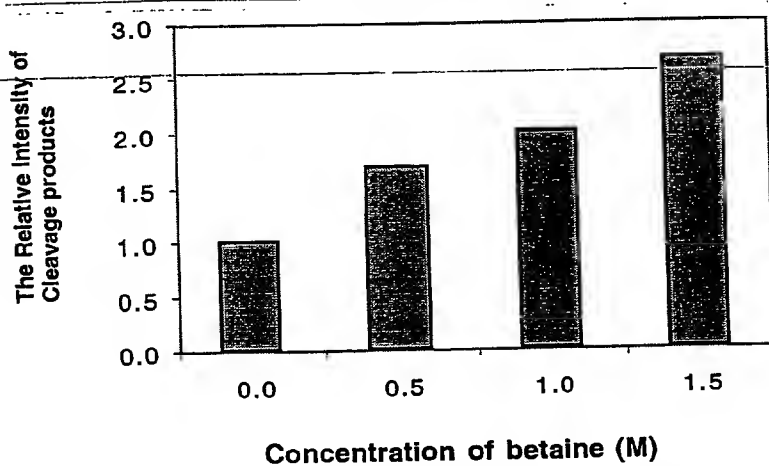


Figure 10

Table 1 Characteristics of the study population

Characteristic	Number of patients	Percentage
Age (years)		
< 60	10	10.0
60-70	10	10.0
71-80	10	10.0
> 80	10	10.0
Gender		
Male	10	10.0
Female	10	10.0
Duration of disease (years)		
< 10	10	10.0
10-20	10	10.0
> 20	10	10.0
Site of disease		
Upper extremities	10	10.0
Lower extremities	10	10.0
Site of surgery		
Open	10	10.0
Arthroscopic	10	10.0
Postoperative treatment		
Physical therapy	10	10.0
Medication	10	10.0
Follow-up (months)		
< 6	10	10.0
6-12	10	10.0
> 12	10	10.0

A

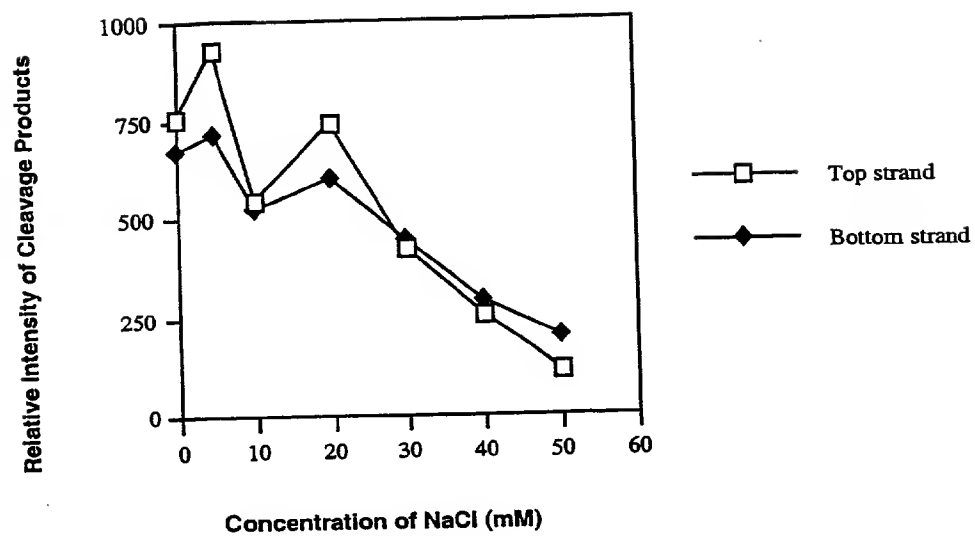
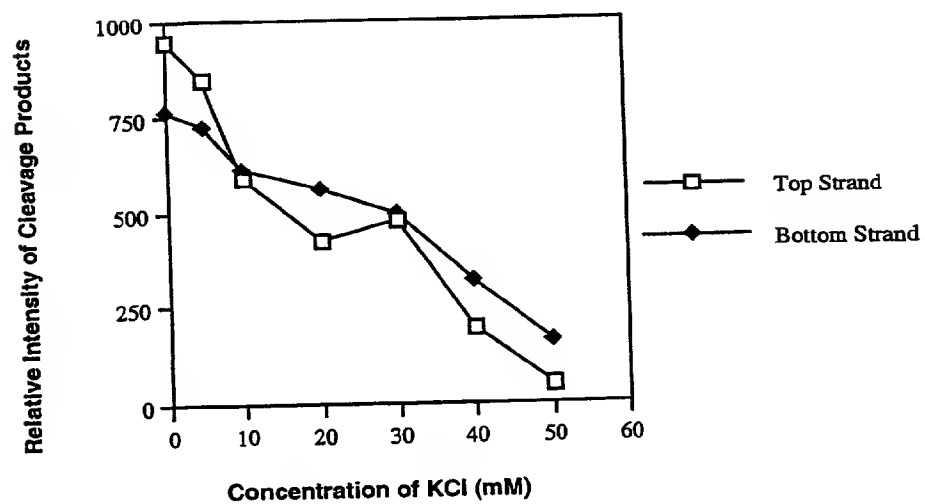
**B**

Figure 11

***k-ras* G12D (G->A)**

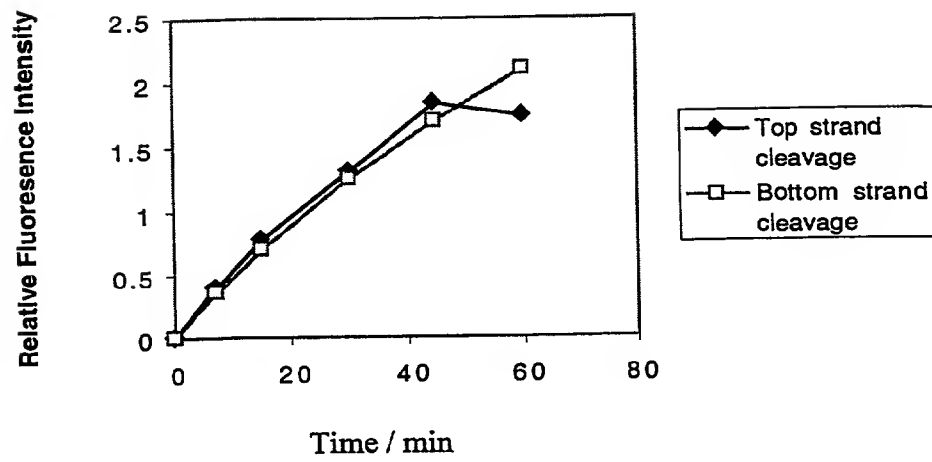
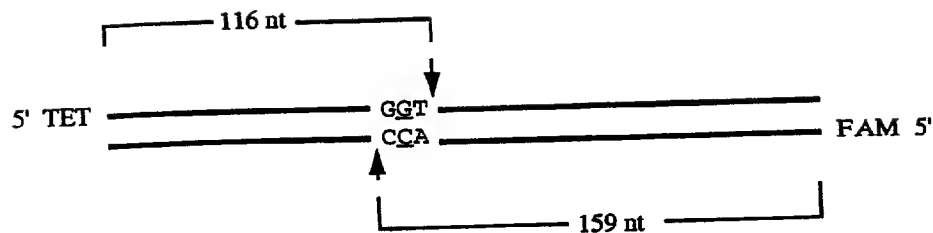


Figure 12

A



B

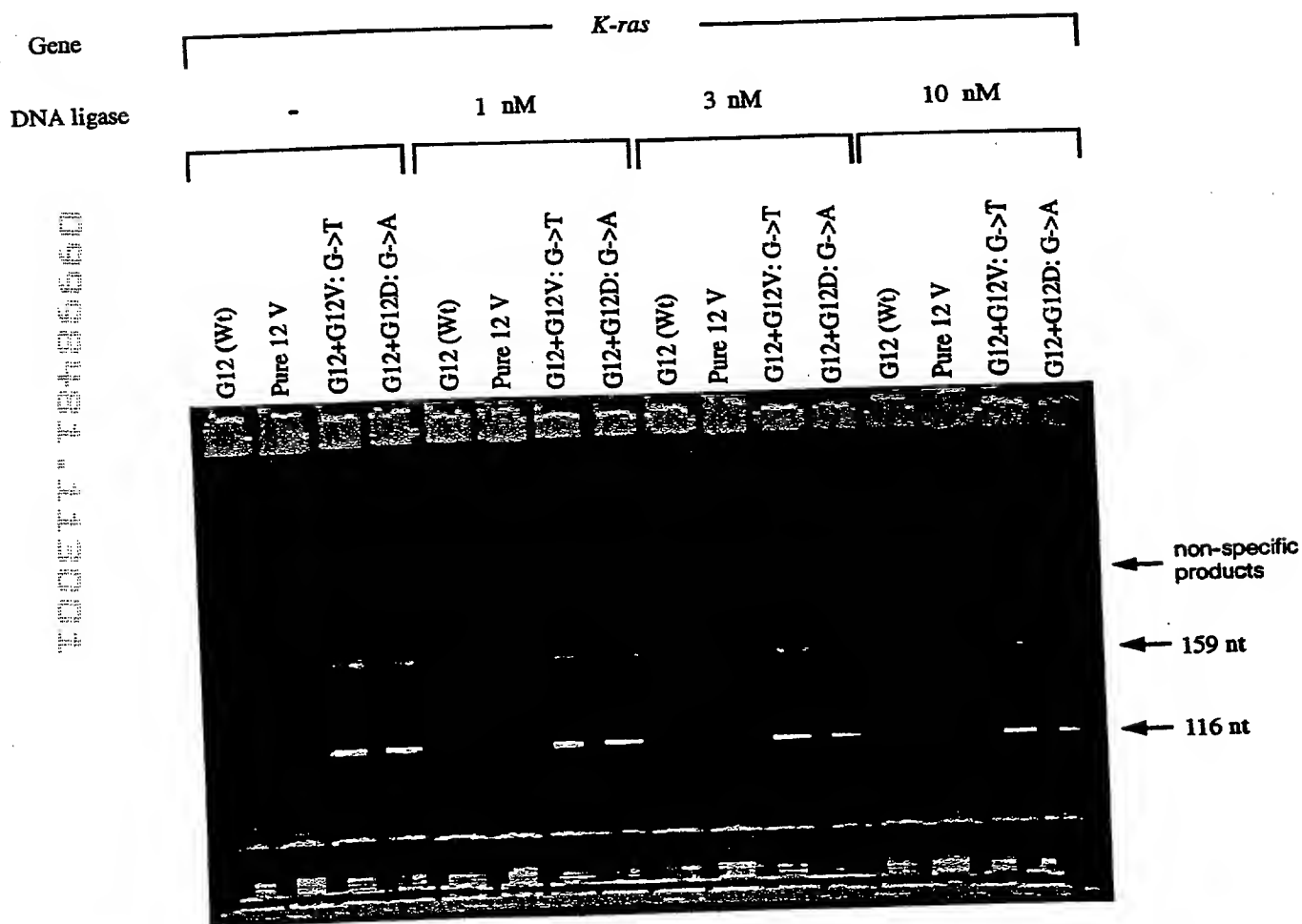


Figure 13

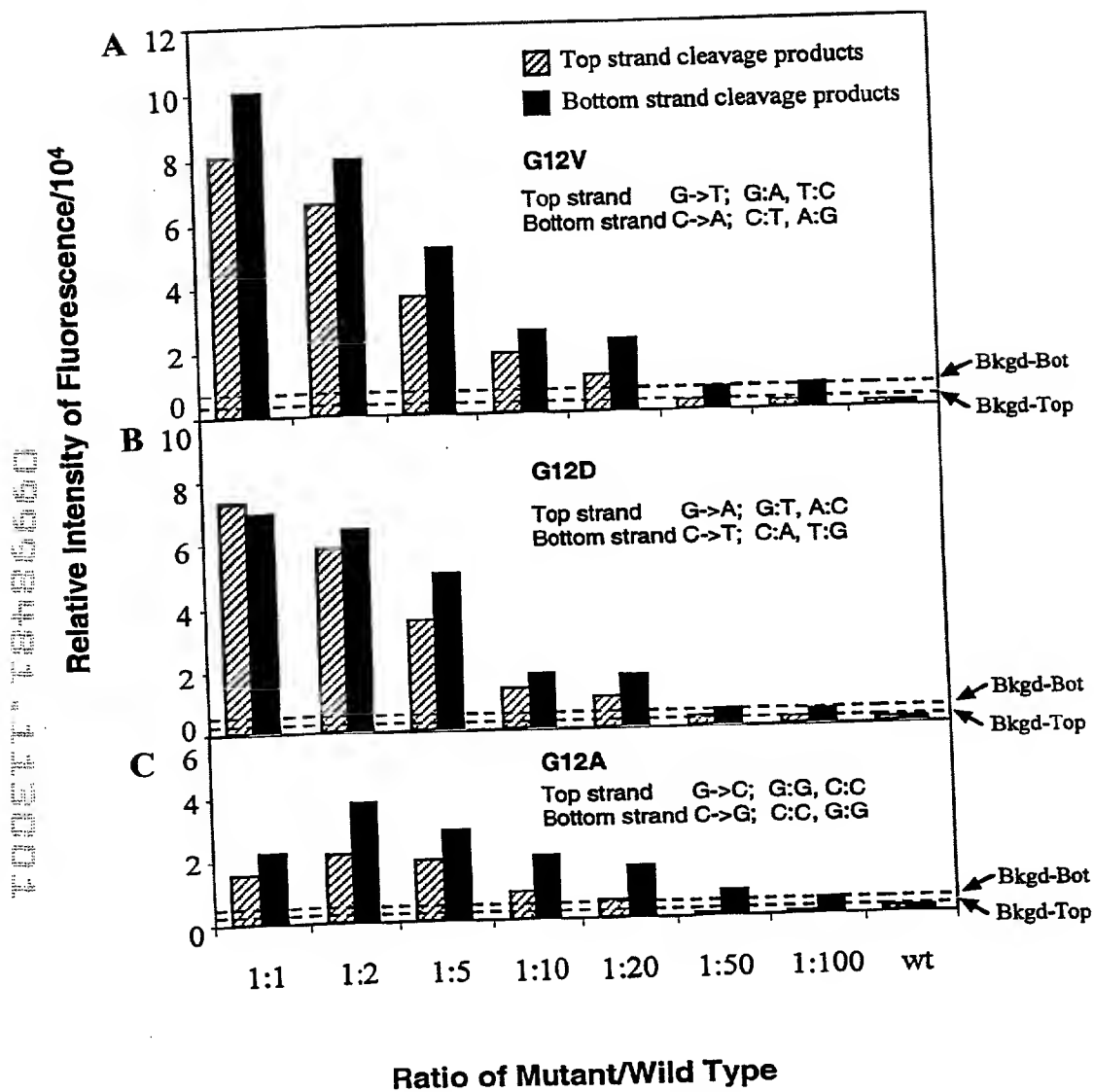


Figure 14

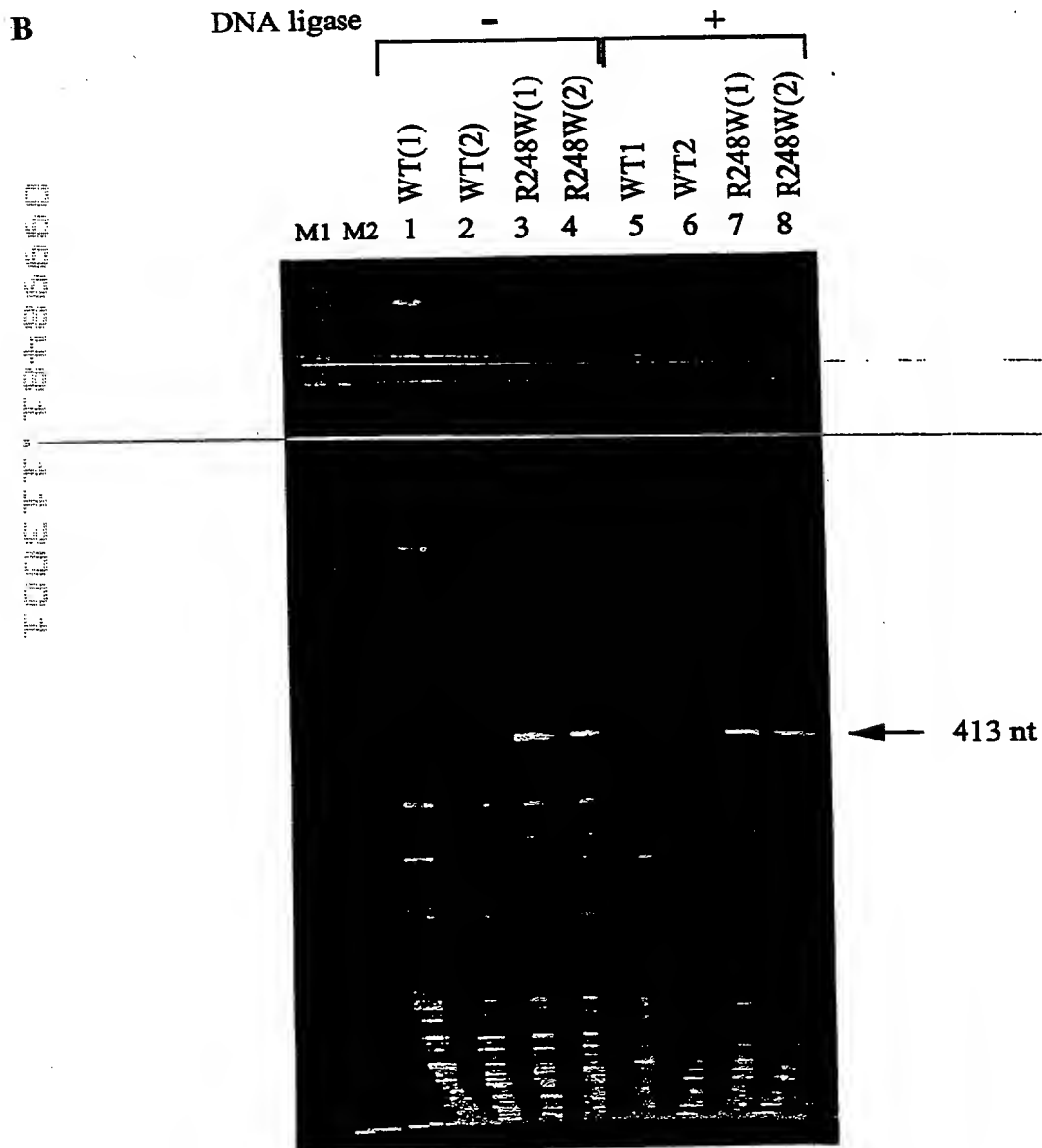
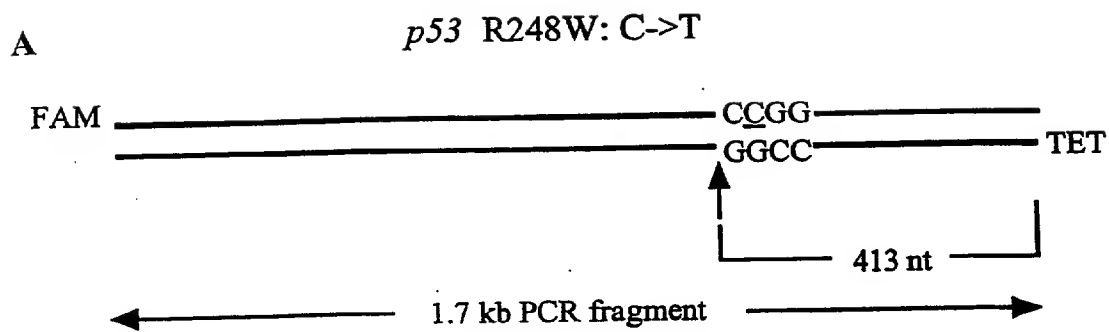
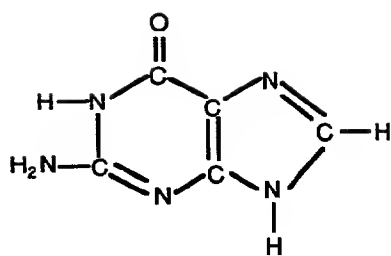


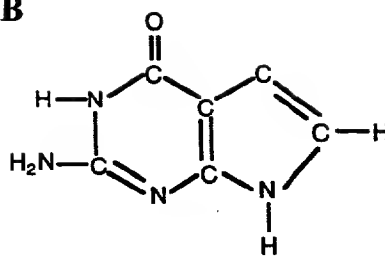
Figure 16

A



Guanine

B



7-deaza-Guanine

09538484.13004
T00577-70485550

C

dG 7deazaG



PCR products

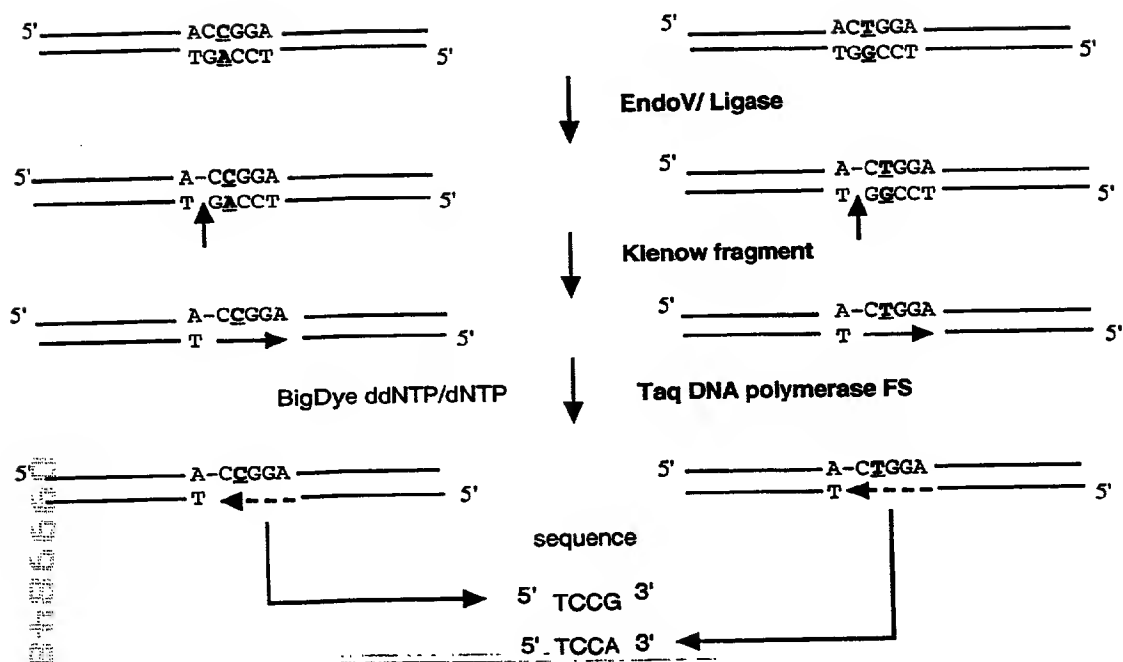
Bottom strand
cleavage
products

Top strand
cleavage
products

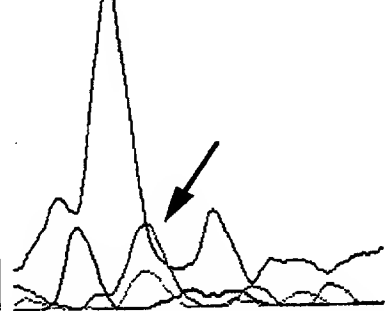
Figure 17

A

p53 R248W(C->T)



B



C C T C C N G T T C A T G C
150

C

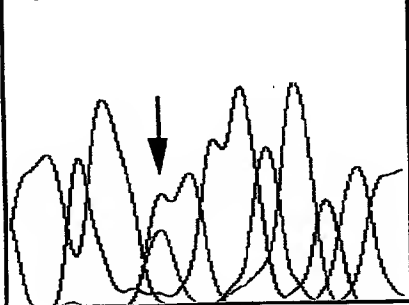
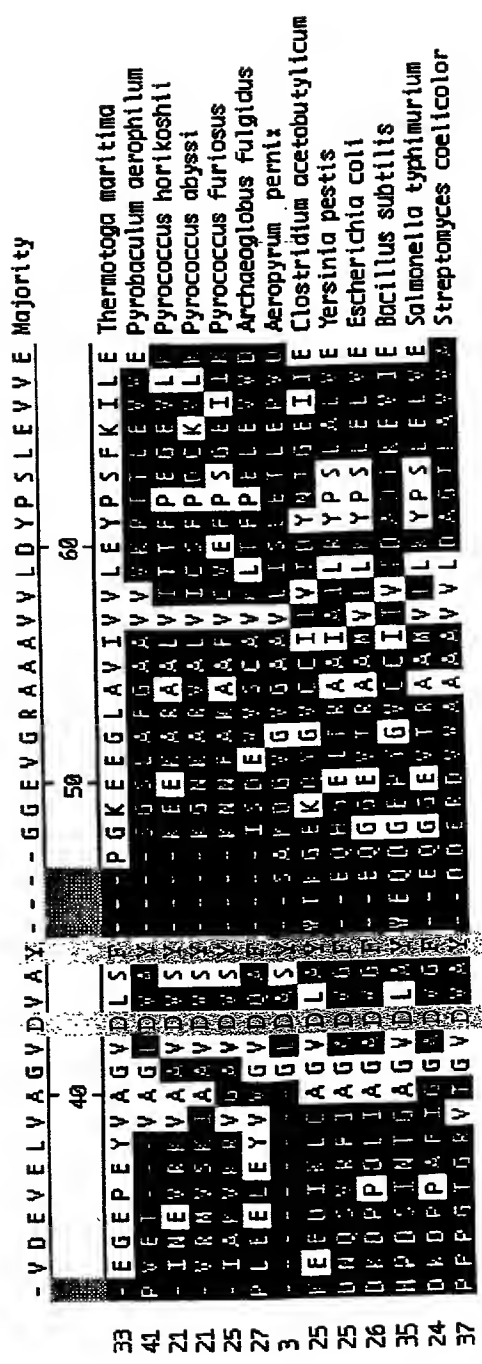
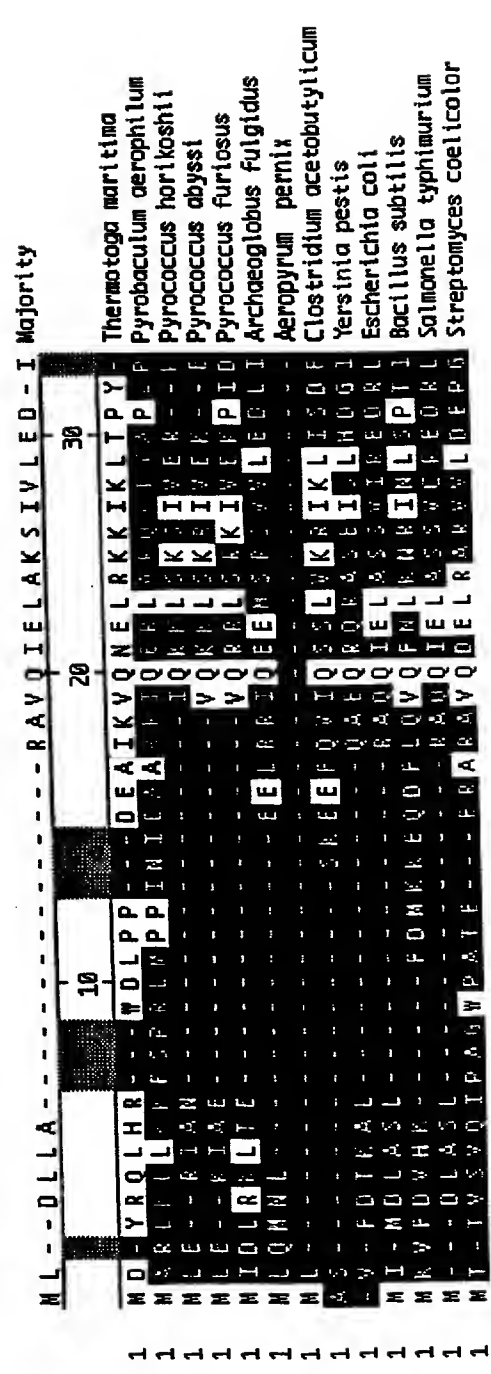


Figure 18



Block I

Figure 19

	TKVAVGRVSFPX	IPGFLAFRE	LPPIIAAWKKLSEEPDVVL	Majority
69	VYSEGEITTFP	IPGLLAFFR	GPPLFLKAWKLR	Thermotoga maritima
74	IALVSTI	IPGLLAFFR	GPPLFLKAWKLR	Pyrobaculum aerophilum
56	IKVIEI	IPGLLAFFR	GPPLFLKAWKLR	Pyrococcus horikoshii
56	IKVLEI	IPGLLAFFR	GPPLFLKAWKLR	Pyrococcus abyssi
60	TKVIEI	IPGLLAFFR	GPPLFLKAWKLR	Pyrococcus furiosus
63	KAVRVEKVTTFP	IPGLLAFFR	GPPLFLKAWKLR	Archaeoglobus fulgidus
32	ERVYISLVLLIP	IPGLLAFFR	GPPLFLKAWKLR	Aeropyrum pernix
65	KAYDGEIEVPP	IPGLLAFFR	GPPLFLKAWKLR	Clostridium acetabutylicum
63	YQVARRATSLPP	IPGLLAFFR	GPPLFLKAWKLR	Yersinia pestis
64	YKVARRTATIMPP	IPGLLAFFR	GPPLFLKAWKLR	Escherichia coli
75	KVHSMGRITMPP	IPGLLAFFR	GPPLFLKAWKLR	Bacillus subtilis
62	YKVARRTATIMPP	IPGLLAFFR	GPPLFLKAWKLR	Salmonella typhimurium
75	EATAVGRITMPP	IPGLLAFFR	GPPLFLKAWKLR	Streptomyces coelicolor

Block II

	V D G H G I A H P R R L G L A S H I G L L L G K P T I G V A K S R L C G T	Majority
109	F D G G L A H P R R K L G I A S H M G L F I E I P T I G V A K S R L Y G T	Thermotoga maritima
114	V D G H G I A H P R R L G L A S H M G L F I E I P T I G V A K S R L Y G E	Pyrobaculum aerophilum
94	V D G H G I A H P R R L G L A S H M G L F I E I P T I G V A K S R L Y G E	Pyrococcus horikoshii
94	V D G H G I A H P R R L G L A S H M G L F I E I P T I G V A K S R L Y G E	Pyrococcus abyssi
98	V D G H G I A H P R R L G L A S H M G L F I E I P T I G V A K S R L Y G E	Pyrococcus furiosus
103	V D G H G I A H P R R L G L A S H M G L F I E I P T I G V A K S R L Y G E	Archaeoglobus fulgidus
72	V D G H G I A H P R R L G L A S H M G L F I E I P T I G V A K S R L Y G E	Aeropyrum pernix
105	F D G G L A H P R R K L G I A S H M G L F I E I P T I G V A K S R L Y G T	Clostridium acetabutylicum
103	V D G G L A H P R R K L G I A S H M G L F I E I P T I G V A K S R L Y G U	Yersinia pestis
104	V D G G L A H P R R K L G I A S H M G L F I E I P T I G V A K S R L Y G U	Escherichia coli
115	F D G G L A H P R R K L G I A S H M G L F I E I P T I G V A K S R L Y G U	Bacillus subtilis
102	V D G G L A H P R R K L G I A S H M G L F I E I P T I G V A K S R L Y G U	Salmonella typhimurium
115	V D G G L A H P R R K L G I A S H M G L F I E I P T I G V A K S R L Y G U	Streptomyces coelicolor

Block III

Block IV

Figure 19 (cont.)

F--LED--GA--P LLDGGEGQLGYVLR TK-RCKPI--RVSVG Majority											
150 160 170											
146	FK	MP	ED	KR	CS	WS	YL	YD	GE	E I G C V I R T K E G S A P I - F V S P G	Thermotoga maritima
151	-	-	-	-	-	-	-	-	-	-	Pyrobaculum aerophilum
125	-	-	-	-	-	-	-	-	-	-	Pyrococcus horikoshii
125	-	-	-	-	-	-	-	-	-	-	Pyrococcus abyssi
129	-	-	-	-	-	-	-	-	-	-	Pyrococcus furiosus
140	WV	EV	ED	-	-	-	-	-	-	-	Archaeoglobus fulgidus
109	-	-	-	-	-	-	-	-	-	-	Aeropyrum pernix
145	FEM	PE	S	FE	DA	FK	U	TV	INE	E	Clostridium acetobutylicum
140	FEL	PH	QD	WG	GA	VQ	PL	FD	MU	E	Yersinia pestis
141	FEL	PS	EP	GA	LA	PL	MD	KG	EL	A	Escherichia coli
155	FW	TE	PE	IE	WG	AY	TD	IT	OG	E	Bacillus subtilis
139	FE	PL	SA	EP	GA	LS	PL	MD	PG	E	Salmonella typhimurium
152	DD	PD	TY	PR	GS	TS	PL	LA	GA	E	Streptomyces coelicolor

Block V

H R I T L D S A L A I V Q A L - L D - - G Y R L P E P T R L A D A L A K - R - - Majority																																								
190 200 210 220																																								
185	H	L	M	D	V	E	S	S	K	R	L	I	K	A	F	T	L	P	-	G	R	R	I	P	E	P	T	R	L	A	H	-	I	Y	T	Q	R	L	K	Thermotoga maritima
181	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pyrobaculum aerophilum	
147	N	L	I	T	L	E	D	A	T	E	I	I	R	A	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pyrococcus horikoshii		
147	N	M	I	T	L	E	D	A	V	R	I	I	E	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pyrococcus abyssii		
151	N	L	I	T	L	E	D	A	V	R	I	I	E	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pyrococcus furiosus		
176	V	I	S	P	D	S	A	L	E	L	-	T	R	E	C	L	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Archaeoglobus fulgidus		
142	H	R	I	T	L	E	D	A	T	E	I	I	R	A	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Aeropyrum pernix		
184	H	R	I	T	L	E	D	A	T	E	I	I	R	A	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Clostridium acetobutylicum		
179	H	R	V	S	V	D	S	A	L	A	V	V	Q	R	C	-	M	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Yersinia pestis		
180	H	R	V	S	V	D	S	A	L	A	V	V	Q	R	C	-	M	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Escherichia coli		
194	W	Y	I	D	L	D	S	S	T	Q	I	T	M	S	-	-	L	I	N	Q	E	S	R	L	P	I	P	V	R	L	A	D	-	-	-	-	-	-	Bacillus subtilis	
178	H	R	V	S	T	D	S	A	L	A	V	V	Q	R	C	-	M	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Salmonella typhimurium		
191	H	R	V	G	L	G	A	E	A	H	T	L	A	L	-	-	T	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Streptomyces coelicolor		

Block VI

Figure 19 (cont.)

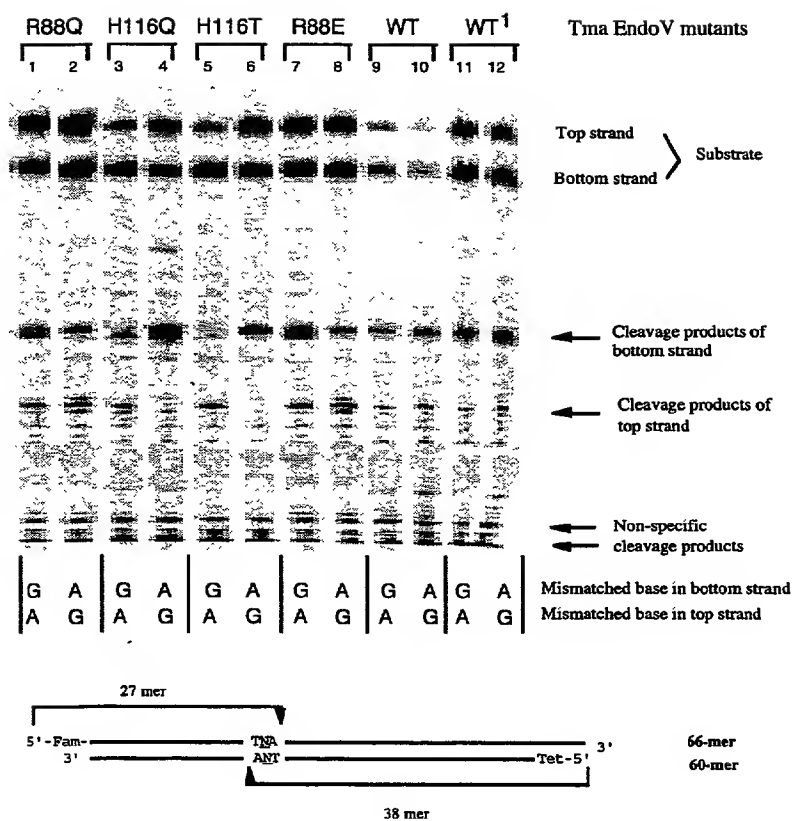


Figure 20

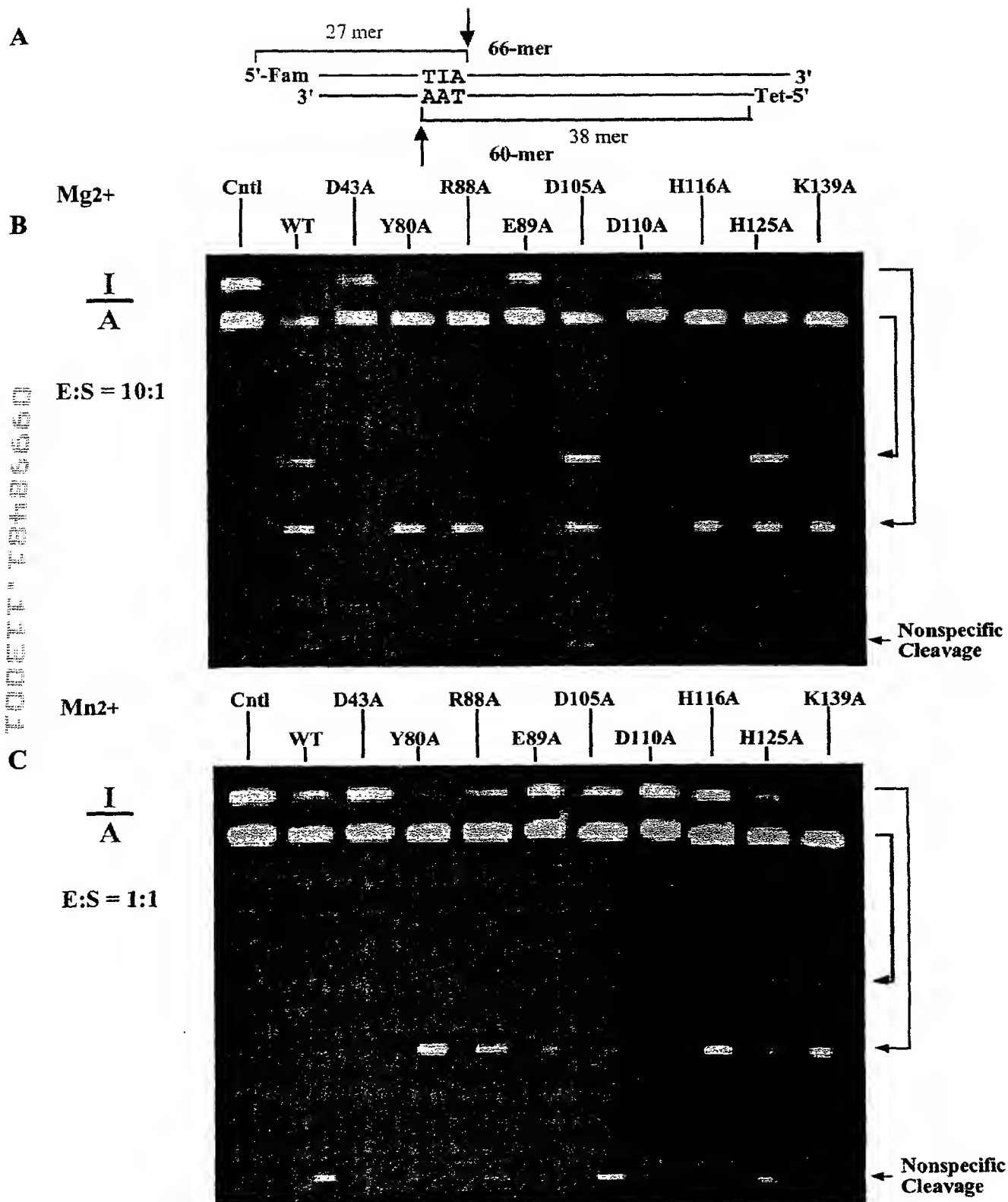


Figure 21

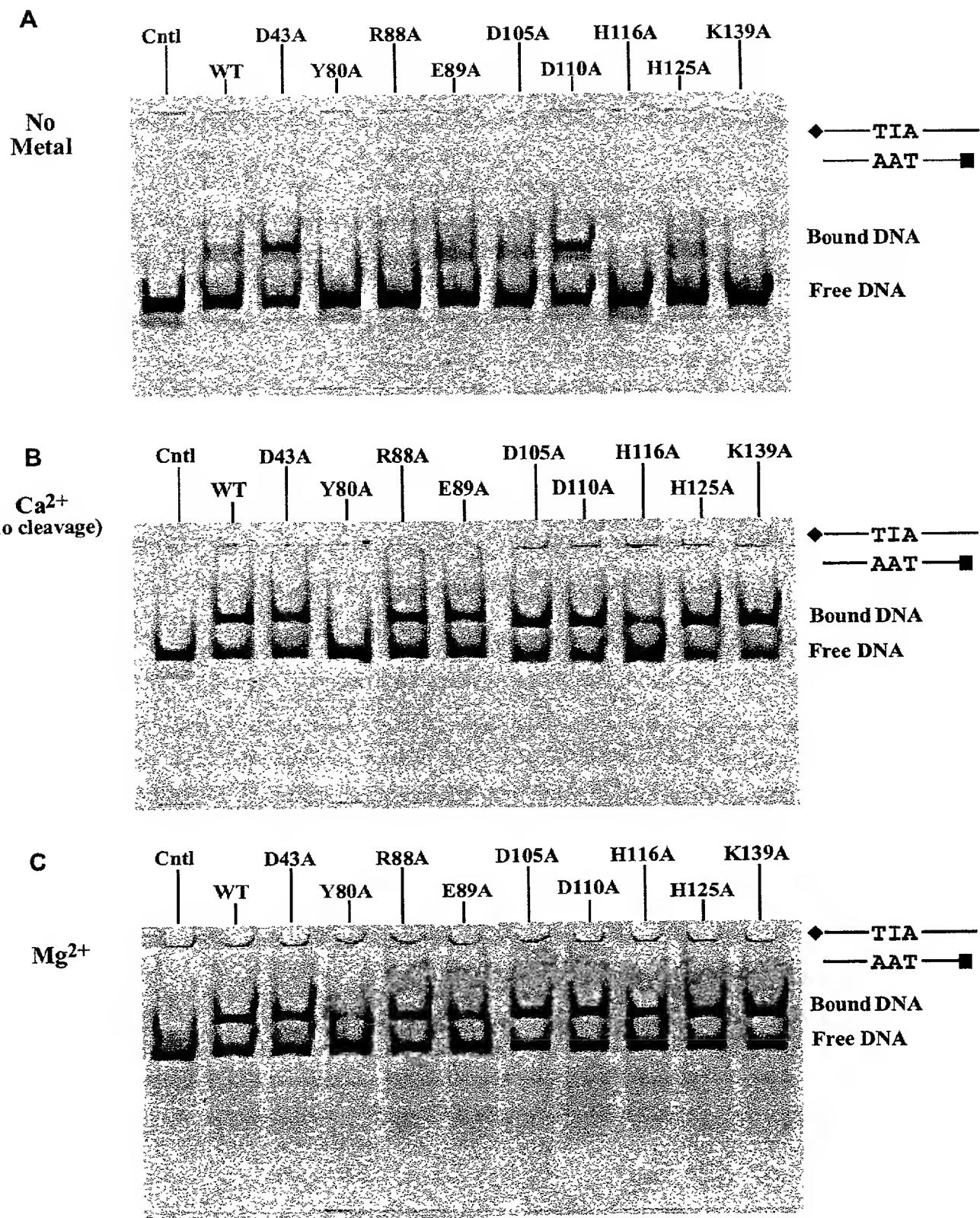


Figure 22

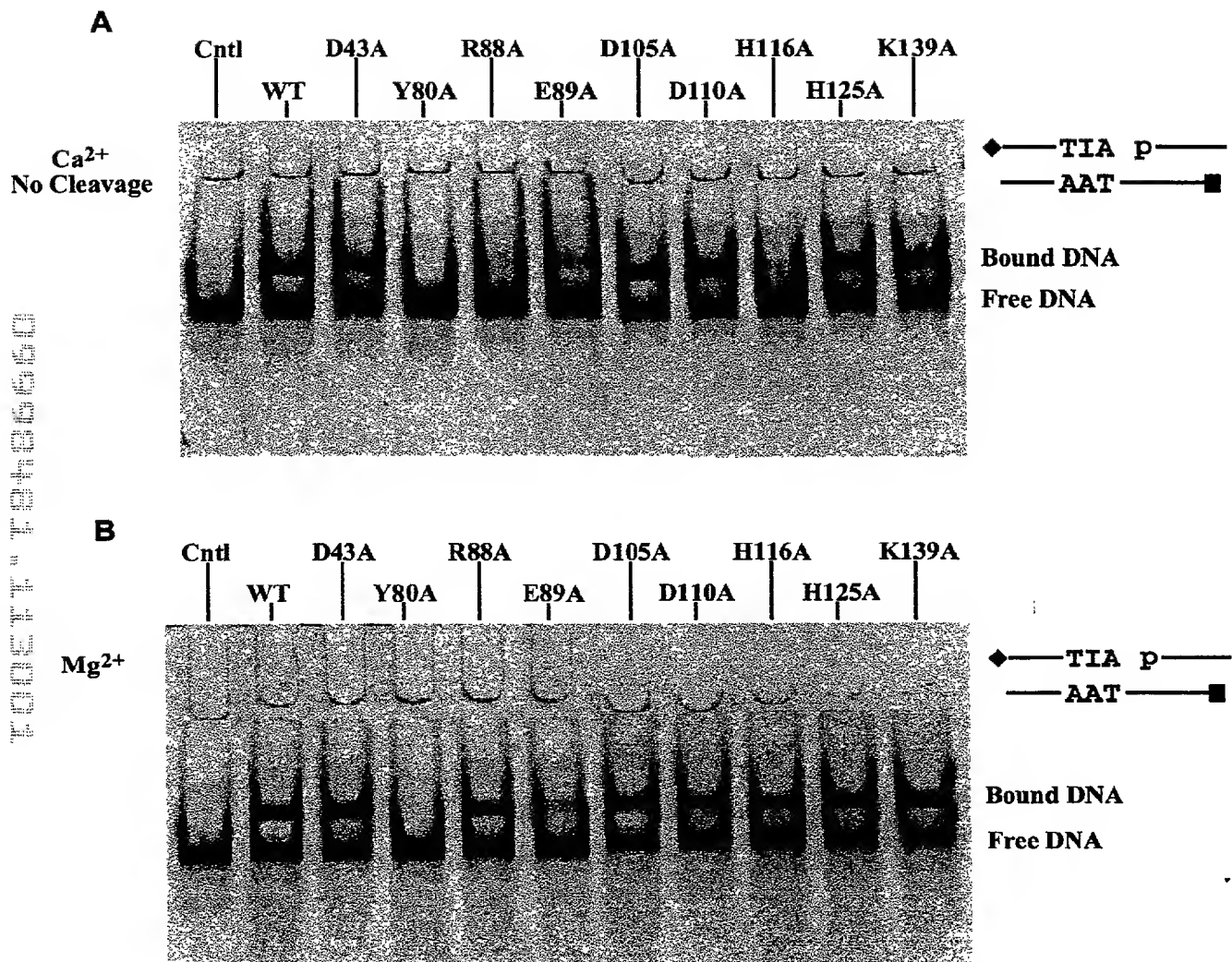
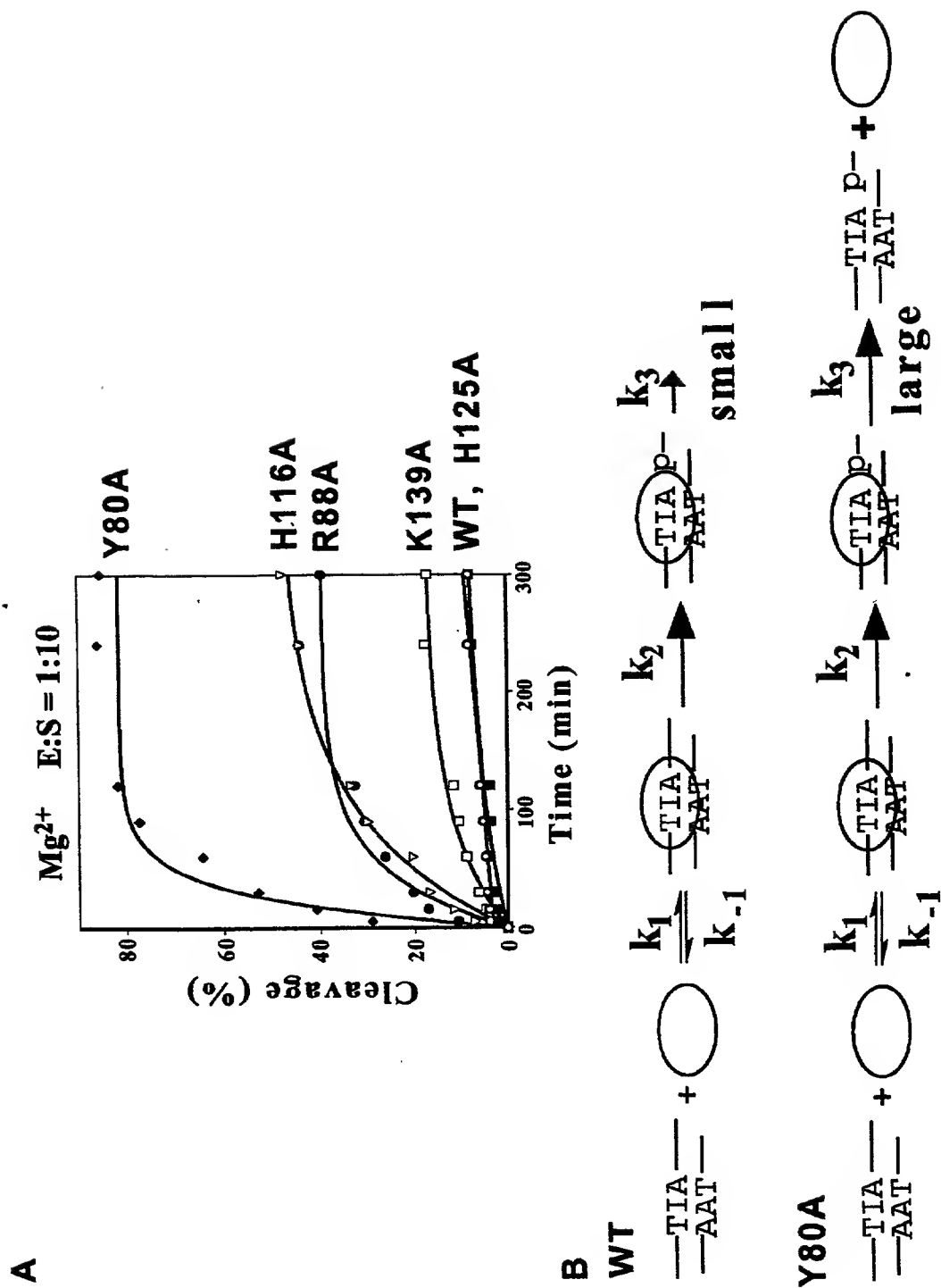
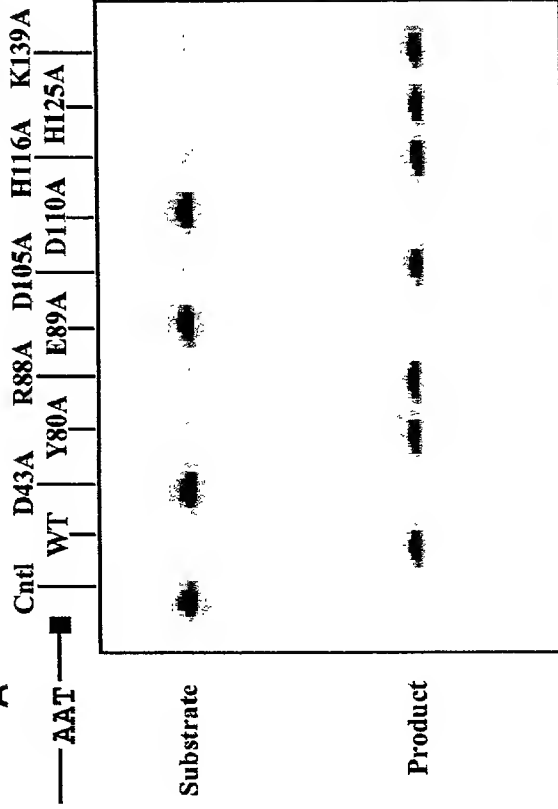


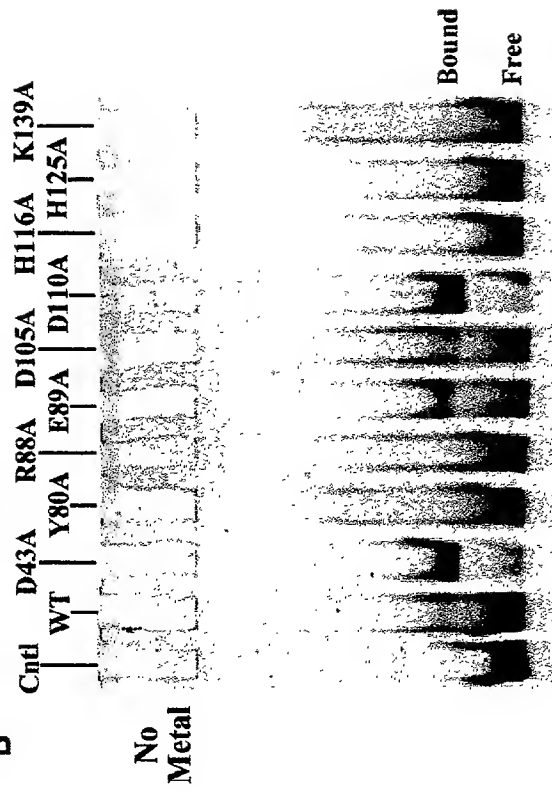
Figure 23



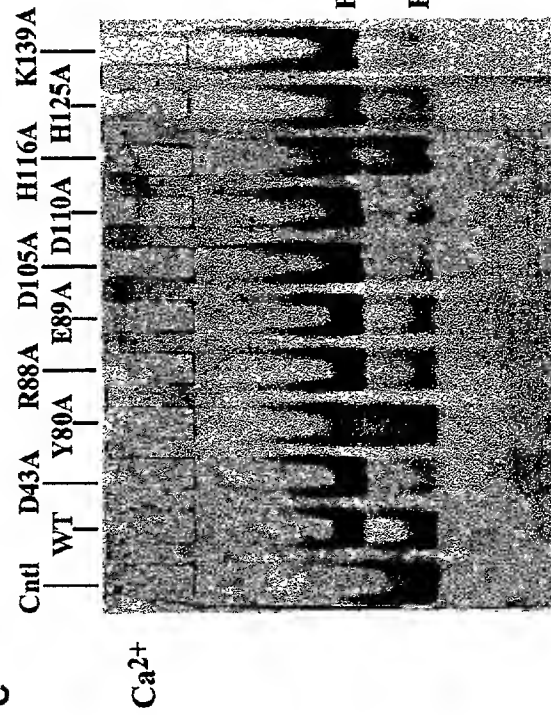
A



B



C



D

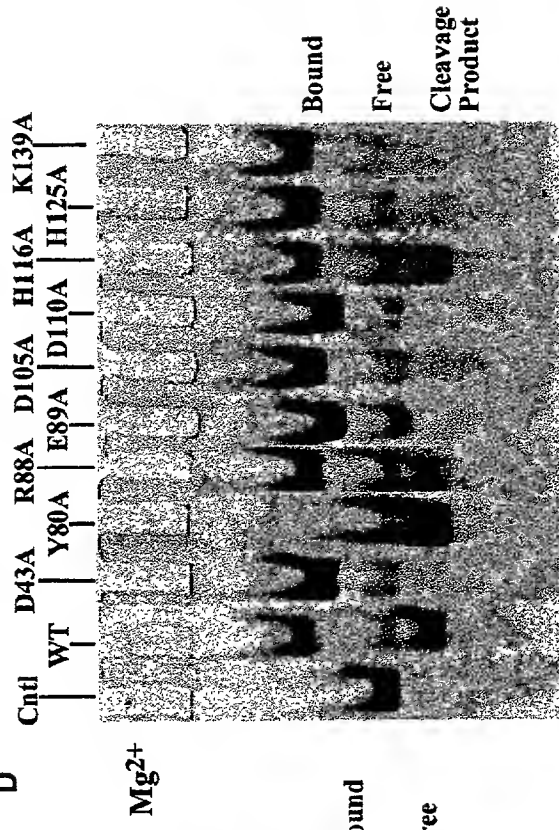


Figure 25

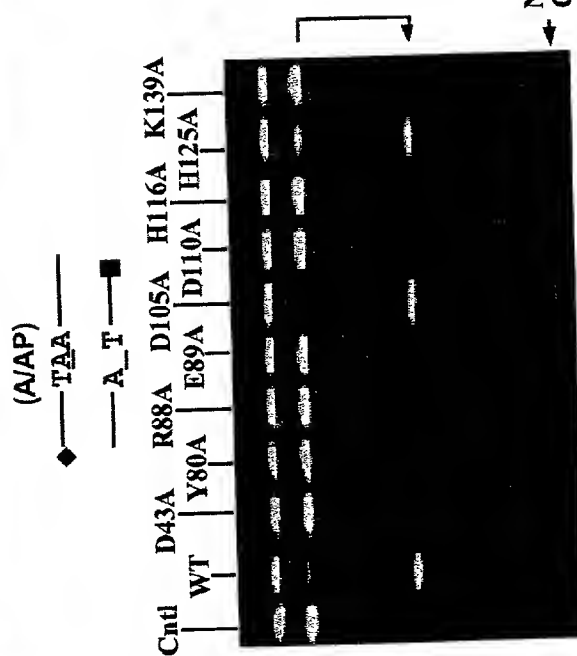
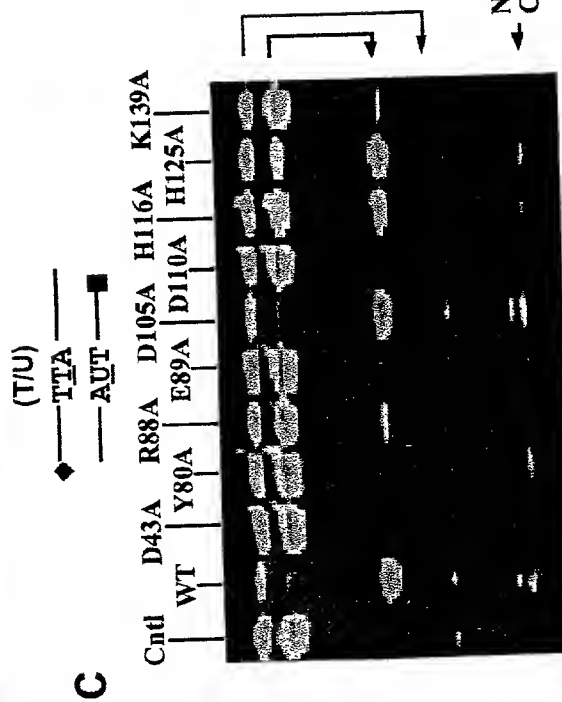
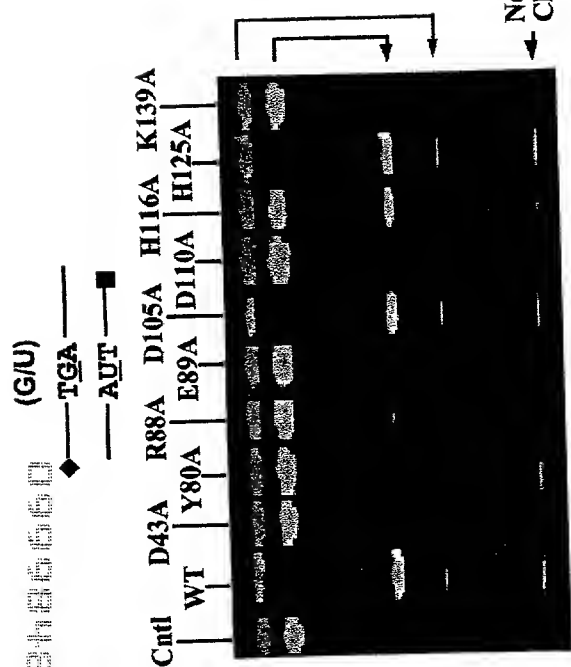
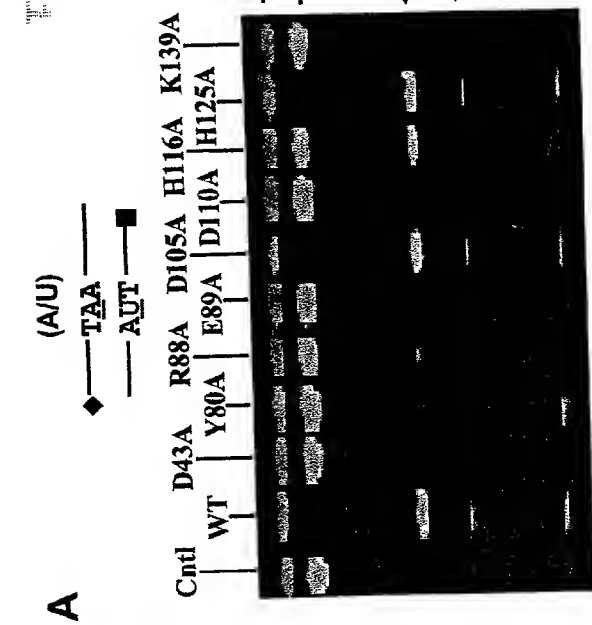


Figure 26